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# Relaxin and Estrogen Accelerate Growth of Uterine Cervix of Prepubertal Pigs

## Abstract

One of the primary targets for the biological actions of relaxin is the uterine cervix. The effects of relaxin on this organ include inducing growth and remodeling of connective tissue and cervical cells to accommodate pregnancy and parturition. The related physiological and clinical observations include softening, ripening, and dilating the cervix and facilitating delivery of the young. The underlying molecular and cellular mechanisms remain obscure. Collagen is the most abundant protein in the cervix and plays a key role in connective tissue remodeling. This study was designed to determine the developmental and growthpromoting effects of relaxin with or without estrogen on the uterine cervix of prepubertal gilts. Littermate gilts of 80 days old were randomly assigned to four treatments: vehicle (control, 1 milliliter [ml.] phosphate buffer saline [PBS] and 1 ml. vegetable oil, n = 5); relaxin (relaxin, in PBS, 1 ml., 167 mg. ml.<sup>-1</sup>, n = 5); estradiol benzoate (EB, in vegetable oil, 1 ml., 2 mg. ml.<sup>-1</sup>, n = 5); and relaxin plus EB (relaxin + EB, at the same doses, n = 5), total six intramuscular injections for all treatments. Twenty-four hours after the last injection, the uterus was surgically removed, and the uterine tissues were immediately frozen at -80°C. Homogenates of uterine horns and cervixes were analyzed for concentrations and contents of protein or hydroxyproline (collagen index) and DNA. Relaxin alone had no significant effect on wet or dry weight of the uterus. EB alone increased significantly ( $P < .05$ ) wet weight of the uterus. In the presence of EB, relaxin treatment increased all measurements compared with control (i.e., wet weight). Compared with EB, Relaxin + EB significantly ( $P < .05$ ) increased the uterine wet weight, the hydroxyproline content and DNA content. These results indicate that the growth-promoting effects of relaxin on the uterus and cervix may be, at least partly, estrogen-dependent and that the growth and development of the uterus and cervix can be accelerated by a combination of relaxin and estrogen treatment. The present results show that the growth and development of the porcine uterus and cervix can be accelerated by treatment with relaxin and estrogen. Although it is not known whether the pig at this prepubertal stage contains relaxin receptors after relaxin and estrogen stimulation, the results reported here indicate that relaxin regulates the growth and development of the uterus and cervix during pregnancy as well as during the prepubertal period.

## Keywords

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## Disciplines

Agriculture | Animal Sciences

# Relaxin and Estrogen Accelerate Growth of Uterine Cervix of Prepubertal Pigs

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## Summary and Implications

One of the primary targets for the biological actions of relaxin is the uterine cervix. The effects of relaxin on this organ include inducing growth and remodeling of connective tissue and cervical cells to accommodate pregnancy and parturition. The related physiological and clinical observations include softening, ripening, and dilating the cervix and facilitating delivery of the young. The underlying molecular and cellular mechanisms remain obscure. Collagen is the most abundant protein in the cervix and plays a key role in connective tissue remodeling. This study was designed to determine the developmental and growth-promoting effects of relaxin with or without estrogen on the uterine cervix of prepubertal gilts.

Littermate gilts of 80 days old were randomly assigned to four treatments: vehicle (control, 1 milliliter [ml.] phosphate buffer saline [PBS] and 1 ml. vegetable oil,  $n = 5$ ); relaxin (relaxin, in PBS, 1 ml.,  $167 \mu\text{g. ml.}^{-1}$ ,  $n = 5$ ); estradiol benzoate (EB, in vegetable oil, 1 ml.,  $2 \text{ mg. ml.}^{-1}$ ,  $n = 5$ ); and relaxin plus EB (relaxin + EB, at the same doses,  $n = 5$ ), total six intramuscular injections for all treatments. Twenty-four hours after the last injection, the uterus was surgically removed, and the uterine tissues were immediately frozen at  $-80^{\circ}\text{C}$ . Homogenates of uterine horns and cervixes were analyzed for concentrations and contents of protein or hydroxyproline (collagen index) and DNA. Relaxin alone had no significant effect on wet or dry weight of the uterus. EB alone increased significantly ( $P < .05$ ) wet weight of the uterus. In the presence of EB, relaxin treatment increased all measurements compared with control (i.e., wet weight). Compared with EB, Relaxin + EB significantly ( $P < .05$ ) increased the uterine wet weight, the hydroxyproline content and DNA content. These results indicate that the growth-promoting effects of relaxin on the uterus and cervix may be, at least partly, estrogen-dependent and that the growth and development of the uterus and cervix can be accelerated by a combination of relaxin and estrogen treatment.

The present results show that the growth and development of the porcine uterus and cervix can be accelerated by treatment with relaxin and estrogen. Although it is not known whether the pig at this prepubertal stage contains relaxin receptors after relaxin and estrogen stimulation, the results reported here indicate that relaxin regulates the growth and development of the uterus and cervix during pregnancy as well as during the prepubertal period.

## Introduction

Relaxin is a pregnancy-associated polypeptide hormone. Its primary target organs seem to be the components of reproductive system such as the uterus and cervix. The main

physiological actions of relaxin are to prepare the birth canal and to facilitate parturition. The specific effects of this hormone include growth-promotion of the uterus and cervix, suppression of uterine contractile activity and dilatation of the uterine cervix. Most of these biological effects are estrogen-dependent. In pigs, relaxin has been reported to stimulate the growth of the uterus, cervix, and mammary glands. Whether these effects of relaxin on uterine tissues are estrogen-dependent or just augmented by estrogen is not fully known. The present study was designed to determine the effects of relaxin alone or in conjunction with estrogen on the growth and modification of uterine connective tissue of prepubertal gilts by measuring the concentration and contents of DNA, protein and collagen in the tissues.

## Materials and Methods

### *Animals and hormone treatments*

Purebred Yorkshire gilts, 80 days old, were housed indoors under controlled light and temperature conditions during the experimental period. There were four treatment groups: control (vehicle, 1 ml. PBS and 1 ml. vegetable oil,  $n = 5$ ); relaxin (relaxin, 1 ml.,  $167 \mu\text{g. ml.}^{-1}$ ,  $n = 5$ ); estradiol benzoate (EB, 1 ml.,  $2 \text{ mg. ml.}^{-1}$ ,  $n = 5$ ); and relaxin plus EB (relaxin + EB, at the same doses,  $n = 5$ ). All treatments were administered by intramuscular injection every other day for two weeks (total six injections). To eliminate variation from different litters, four littermates at similar body weight from the same litter were randomly assigned to the four treatment groups in each experiment. Hysterectomy was performed 24 hours after the last hormone or placebo injection and fresh weights (wet weight) of the uterine horns and uterine cervix were determined. A 2-cm. wide ring of the middle portion of the uterine (the location with equal distance from each end of the uterine horn) and a 2-cm. wide ring from the middle portion of the cervix (the location with equal distance from the internal and external cervical os) were excised and weighed; one was immediately frozen at  $-80^{\circ}\text{C}$  until assayed for DNA, protein and hydroxyproline contents, and the other was dried to a constant weight in an oven at  $100^{\circ}\text{C}$  to determine the dry weight and water content.

### *DNA determination*

DNA concentrations and contents in tissue homogenates were determined in duplicate, and calf thymus DNA was used as an assay standard. A DNA fluorescent dye (H33258, final concentrations of  $1.0 \mu\text{g. ml.}^{-1}$ ) was used to determine relative fluorescence; sensitivity of the assay was  $5 \text{ ng. DNA ml.}^{-1}$ .

### *Protein assay*

The protein contents in tissue homogenates were determined in duplicate spectrophotometrically by using an acidic solution of coomassie brilliant blue G-250.

### *Hydroxyproline determination*

The homogenates of the uterine horn and cervix were analyzed in duplicate for hydroxyproline concentration and content. Acid hydrolysates of tissue homogenates were dried down, and 1.0 ml. H<sub>2</sub>O was added to each tube and standard tubes (1.0 ml., concentrations: 0, .5, 1.0, 1.5, 2.0, 3.0, 5.0, 7.5 and 15.0 hydroxyl-L-Proline  $\mu\text{g. ml.}^{-1}$ ), that included .01 M CuSO<sub>4</sub>, 2.5 M NaOH, and 30% H<sub>2</sub>O<sub>2</sub>. After five minutes, 100  $\mu\text{l.}$  FeSO<sub>4</sub> (.05 M) and 3 ml. rho-dimethylamino benzaldehyde were added to each tube, and absorbance was measured at 550 nM. The standard curve was linear to 15  $\mu\text{g.}$  and sensitive to .5  $\mu\text{g. ml.}^{-1}$ .

#### *Statistical analysis*

All samples related to DNA, hydroxyproline and protein concentrations were measured in the same time in each type of assay; there was no interassay variance to report. The interaction of treatment by litter was used as the error term to test significance.

### **Results and Discussion**

The data for wet weight and dry weight of the uterus and cervix are presented in Table 1. Relaxin alone did not increase either the wet weight or dry weight of the uterus. Estrogen or relaxin plus estrogen significantly increased ( $P<.05$  and  $P<.01$ , respectively) wet weight and dry weight of the uterus compared with control. Compared with estrogen alone, the relaxin plus estrogen significantly ( $P<.05$ ) stimulated the growth of the uterus; this effect can be viewed as a relaxin effect being estrogen-dependent or a synergistic interaction between relaxin and estrogen. Relaxin alone did not significantly increase the wet weight or dry weight of the cervix. Estrogen or relaxin plus estrogen increased ( $P<.05$  and  $P<.01$ , respectively) wet weight and dry weight of the cervix compared with those of the controls. Relaxin alone had no effect on DNA, protein, hydroxyproline contents. Estrogen or relaxin plus estrogen stimulated significantly ( $P<.05$  and  $P<.01$ , respectively) protein, hydroxyproline and DNA contents compared with those of controls. The synergistic interaction between relaxin and estrogen was significant ( $P<.05$ ) on cervical protein.

The present results indicate that the growth and development of the uterus and cervix of the prepubertal pigs can be accelerated by treatment with relaxin combined with estrogen. After two weeks of sequential injections with relaxin and estrogen, the uteri of the prepubertal pigs were five-fold heavier than those of vehicle-injected animals. The increased weight is reflected in the increase in DNA, protein, collagen content and dry weight, but not the water content. This indicates that normal growth and development can be stimulated without affecting somatic growth because the body weights at the end of treatment between the treatment and control animals were not different (data not shown). Similar phenomena were seen during precocious puberty when growth of sexual organs was greater than that of other somatic organs. Whether the mechanisms underlying these two events (i.e., accelerated growth of the sexual organs in pathological cases or experimentally induced as in this study) were similar or not is unclear. In our present study, the ratio of protein to DNA (an index of tissue hypertrophy or cell size) was similar between treatment and control groups. The ratios of hydroxyproline to DNA or protein

were not significantly different among the four treatment groups (data not shown). Thus, we conclude that the treatments with estrogen and relaxin induce the proportional growth in uterine tissue.

Short-term treatments with high dosages of relaxin can stimulate water imbibition. It seems that increase in uterine wet weight and circumference may be due to the water imbibition caused by this high dose of relaxin treatment over a short period of the experiment. In contrast, the present study showed that uterine weights, dry weights of the uterus and cervix were significantly increased along with the contents of DNA, protein and hydroxyproline of the uterus (data not shown) and cervix.

The mechanism of the synergistic interaction between relaxin and estrogen is not fully understood, but at least a part of this interaction may result from estrogen's ability to induce an increase in the number of relaxin receptors in the uterine cervical cells as demonstrated in our previous study (Huang et al., 1993).

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### **Reference**

Huang, C.J., Li, Y. and Anderson, L.L., 1993. Stimulation of collagen secretion by relaxin and effect of estrogen on relaxin binding in uterine cervical cells of pigs. *J. Reprod. Fertil.*, **98**:153-158.

**Table 1. Effect of relaxin and estrogen on growth of uterine cervix of prepubertal pigs.**

	No. of pigs	Uterus, g.		Cervix, g.		DNA content, mg./cervix	Protein content, mg./cervix	Hydroxyproline content, mg./cervix
		Wet	Dry	Wet	Dry			
PBS control	5	28	4.4	3.3	.58	2.1	190	13
Relaxin	5	36	5.6	4.1	.72	2.4	244	18
EB	5	85*	14.5*	9.9*	2.2*	5.4*	723*	46*
EB + relaxin	5	136**	22.6**	15.1**	3.3**	8.1**	979**	91**

\*P<.05 compared with PBS control.

\*\*P<.01 compared with PBS control.